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Short communication

Quenching the action of germination stimulants using borax and thiourea, a new method for controlling parasitic weeds: A proof of concept

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ABSTRACT

Broomrapes (*Orobanche and Phelipanche* spp) and witchweeds (*Striga* spp) are the two most devastating parasitic weeds causing enormous losses in agriculture. They have a strong dependence on germination stimulants that are released by the host plants. Knowledge of the structure and bioactivity of these stimulants play an essential role in designing control methods for these weeds. The seeds of the parasites are very minute in size and remain viable in the soil for up to 20 years. Reduction of the weed seed bank has been suggested as an attractive option. Suicidal germination is achieved by applying the germination stimulants to the soil prior to planting crop seedlings or sowing crop seeds, *Orobanche* seeds germinate in absence of the host and as a consequence the germinated seeds die due to lack of nutrients from the host plants. This approach requires a very strict protocol and hence difficult to perform at field levels. Recently, we proposed a novel concept for weed control by decomposing the germination stimulants in soil prior to action. In this paper, this concept is further substantiated by two crucial experiments, namely a study of the phototoxic effect of the reagents used and a demonstration the actual quenching of the germination of seeds of parasitic weeds by applying borax and/or thiourea. The results demonstrate that timely decomposition of stimulation is indeed a feasible method for weed control.

1. Introduction

Broomrapes (*Orobanche and Phelipanche* spp) and witchweeds (*Striga* spp) are the two most devastating parasitic weeds causing enormous losses in agricultural food production in tropical and subtropical areas (Parker, 2009, 2012). Tragically, worldwide the problems with these weeds are increasing leading to a negative impact on the economy of many countries. A typical feature of the life cycle of these weeds is that their seeds have special requirements for germination, namely, after-ripening, pre-conditioning, and an obligate dependence on germination stimulants that are released by the host plants (Awad et al., 2006; Bouwmeester et al., 2003). Knowledge of the structure and bioactivity of these germinating stimulants play an essential role in designing control methods for these weeds. Several natural germination stimulants have been isolated and identified (Zwanenburg and Pospíšil, 2013). It should be noted however, that the host plants produce very minute amounts of stimulants (ca15 pg/plant/day, Humphrey and Beale, 2006) and their isolation and structure determination is quite involving. To date about 15 natural stimulants, collectively called strigolactones (SLs), have been isolated and characterized (Yoneyama et al., 2009; Xie et al., 2010, 2013; Zwanenburg and Pospíšil, 2013). The SLs invariably contain three annelated rings (the ABC scaffold, see Fig. 1) connected with a butenolide (D-ring via an enol ether unit). Typical examples of such naturally occurring germination stimulants are strigol (**1**) (Fig. 1), which is the first isolated and most well known stimulant (Cook et al., 1966; Brooks et al., 1985; Xie et al., 2010), and orobanchol (**2**) (Fig. 1), which is probably the most abundant one (Xie et al., 2010, 2013; Zwanenburg and Pospíšil, 2013). Extensive structure-activity studies revealed that the bioactiphore of the stimulants resides in the CD part of the molecule (Mangnus and Zwanenburg, 1992; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013).

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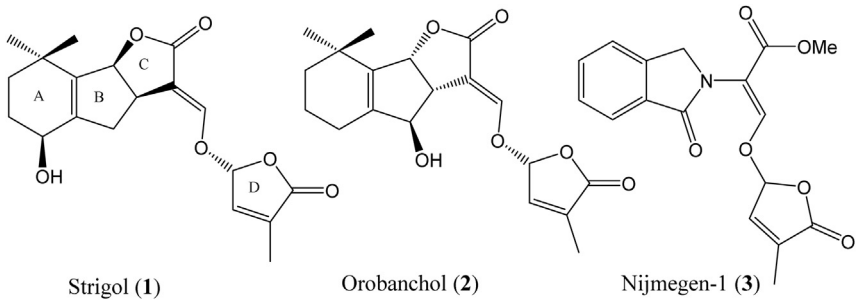

Fig. 1. Structures of some natural SLs and a synthetic SL analog.

Also a tentative molecular mode of action has been proposed (Mangnus and Zwanenburg, 1992; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013). On the basis of this essential molecular information a model for the design of bioactive SL analog was developed (Mangnus and Zwanenburg, 1992; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013).

The GR compounds, e.g. GR24 (structure not show, GR are the initials of the inventor Gerald Rosebery), described by Johnson et al. (1981), are the best known SL analogs. Using the above mentioned model a variety of new SL analogs was designed, prepared and biologically evaluated (Mwakaboko and Zwanenburg, 2011a, 2011b; Zwanenburg et al., 2009; Zwanenburg and Pospíšil, 2013). A good example is Nijmegen-1 (**3**) (Fig. 1), which can readily be obtained and has an appreciable germinating activity (Nefkens et al., 1997).

The parasitic process involves the recognition of the stimulants by the seeds and subsequent germination (Bouwmeester et al., 2003). This evolutionary adaptation of these parasitic plants to respond to the germination stimulants is to ensure that their tiny seeds with limited reserves germinate only in the presence host plants (Butler, 1995). The seeds of these parasites are very minute in size (*Orobanche seeds*: 0.35 by 0.25 mm) and remain viable in the soil for up to 20 years (Puzzilli, 1983; Pacureanu-Joita et al., 2009). When the seeds have germinated, the developing radicle must grow towards the host root and this process is possibly directed by the concentration gradients of germination stimulants (Eastbrook and Yoder, 1998). After attachments to the host root, the essential ingredients for further development of the parasite are made available by the host plant.

In the past four decades several methods for controlling parasitic weeds have been considered (Oswald and Ransom, 2001; Parker, 1991, 2009, 2012; Hearne, 2009; Rodenburg et al., 2010; Rubiales et al., 2009; Hooper et al., 2009; Zwanenburg et al., 2009). Hand weeding is probably the oldest method but it is laborious and not very effective as most of the damage to the host plant has already been done below the surface before the time the parasite can be pulled out. The use of common herbicides, such as glyphosate, requires a very strict regime of application (Castejón-Muñoz et al., 1990; Mesa-García and García-Torres, 1985) with a limited applicability, and is for sure not feasible in the developing countries. Reduction of the seed banks of these weeds has been suggested as an attractive option in the overall successful management of parasitic weeds. This can be achieved, at least in principle, by using the so called suicidal germination approach. By applying a stimulant to the soil prior to planting of the desired crop, seeds will germinate in the absence of a potential host, but as no essential ingredients for further development are available, the germinated seeds will simply die. The first successful mention of this approach dates from 1976 (Johnson et al.). However, these attempts were discontinued due to lack of financial support and problems with the production of sufficient amounts of the synthetic germination stimulant GR7. The suicidal germination approach has been criticized by several authors who claim that the limited stability of SL analogs in the soil, especially in alkaline soil, would preclude their effective use (Johnson et al., 1976; Babiker and Hamdoum, 1982; Babiker et al., 1987, 1988; Rubiales et al., 2009). Recently however, some of the new SL analogs have successfully been used for inducing suicidal germination in pot experiments (Kgosi et al., 2012). In field trials Nijmegen-1 has been used for the control of *Orobanche cumana* in tobacco. In these successful trials the stimulant was formulated in an appropriate emulsifier in order to prevent untimely hydrolysis and leaching down to lower soil layer (Zwanenburg et al., 2009). Due to the strict requirement for its application suicidal germination is not widely used (Rubiales et al., 2009). Judging from the available literature on parasitic weed control, it is evident that there is still a great need for new and effective methods.

Recently, we reported a novel concept for parasitic weed control, namely decomposing the germination stimulants prior to action (Kannan and Zwanenburg, 2014). In this concept, common and simple chemicals, more in particular borax and thiourea, are used to quickly decompose the SLs (and its analogs) and as a consequence seeds of parasitic weeds cannot germinate anymore. In this paper, the concept is further substantiated by two crucial experiments, namely a study of the phytotoxic effect of the reagents used and a demonstration of the actual quenching of the germination of seeds of parasitic weeds by applying borax and/or thiourea.

2. Material and methods

2.1. Orobanche seed collection

Seeds of *Orobanche crenata* were collected from fully matured flowers of *Orobanche* stalks growing around and attached to tomato from the farmer's field near Jabalpur, Madhya Pradesh, India (N 23°24′18″ & E 79°58′30″, 388M) in 2011. The seeds were sun dried and stored in black plastic containers at room temperature (30 ± 2 °C).

2.2. Phytotoxicity studies using borax and/or thiourea on tomato

The experiment was conducted at the containment facility at the Directorate of Weed Science Research, Jabalpur M.P. under controlled conditions. Tomato plants (variety *Pusa rubi*) were grown in 10 kg volume plastic pots. *Orobanche* infested soil was used. Seedlings were allowed to grow for 25 days with subsequent thinning to remove weak plants. Phytotoxicity studies were performed by preparing borax and thiourea solutions of different molar concentrations namely, 100, 50, 25, 10, 5, 1, 0.5 mM, in sterile doubly distilled water. Then 5 ml of these solutions per plant was drenched near the root zone of about 25 days old seedlings of tomato. The phytotoxic effects were observed at different days